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RARE 2-SUBSTITUTED PURINE NUCLEOSIDES

ANNUAL/FINAL REPORT

May 1989

Vasu Nair

Supported by

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19. ABSTRACT (Continue on reverse if necessary and identify by block number) This project was concerned with the synthesis of rare 2-substituted purine nucleosides with therapeutic potential against RNA viruses. A total of twenty-two rare 2-substituted purine nucleosides were synthesized, purified, characterized, and submitted to the Department of Antiviral Studies. Antiviral screening data received to date show some very interesting and positive results. One compound (2-acetonyinosine, AVS-002159) has been found to have very high activity (TI > 1000) against the Sandfly Fever Virus (Phlebovirus). Another compound (2-vinylinosine, AVS-002716) has been found to have low but broad spectrum activity against a number of RNA viruses. Two other compounds (AVS-002883 and AVS-002884) have shown some activity against the Rift Valley Fever Virus, and still another (AVS-002352) has shown activity against the Yellow Fever Virus. 2-Formyinosine (AVS-004094) has shown some activity against Type 2 Adenovirus and 2-hydroxymethylinosine (AVS-004232) has shown activity against the Vaccinia Virus. Ten publications have arisen directly from this work. Keywords; chemical composition

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3. Name of Principal Investigator: Vasu Nair
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University of Iowa
Phone: (319) 335-1364

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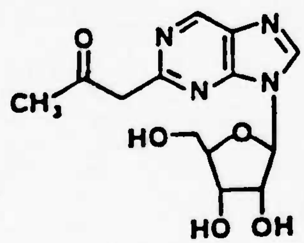
5. Description of Work Accomplished:

The overall goals of the contract were to develop procedures and synthesize, purify, and submit with complete physical data sixteen rare purine nucleosides for antiviral studies. These goals were accomplished. A total of twenty-two novel compounds were submitted to the Department of Antiviral Studies, USAMRIID for biological evaluation. A description of the synthetic work accomplished during the entire period of the award in chronological order, the compounds submitted, the biological screening data, publications, personnel supported, and an executive summary are given in the pages that follow.

In the first year of this contract, our goals were to develop rational procedures for the synthesis of the target molecules discussed in the proposal. Although difficulties were encountered in approaches studied in the first six months of the contract, these problems were entirely overcome and an excellent and novel methodology for the key step in the synthesis of many of the target compounds was discovered.

The starting point of our work during the first year involved the preparation of multigram quantities of some basic precursors and investigation of feasible approaches to the synthesis of 2-acetonylnebularine **1** and 2-acetonylinosine **2**. Target molecules **1** and **2** (in their protected form) were considered to be direct precursors for the synthesis of **3** and **4**, respectively.



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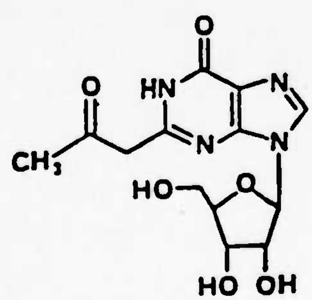

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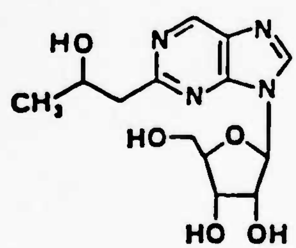

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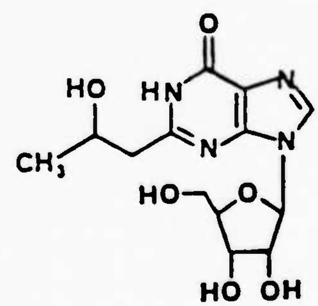
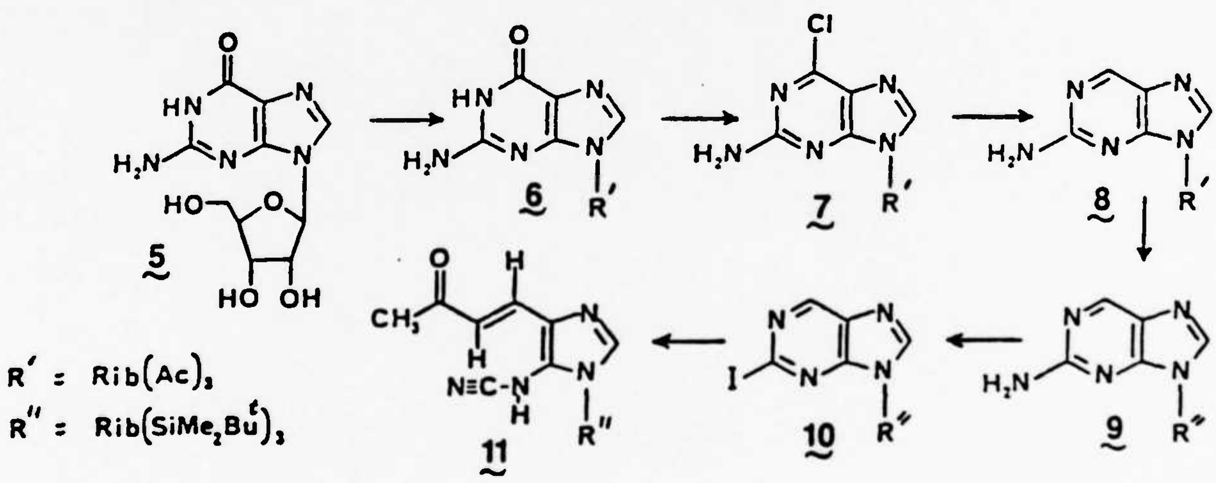


4

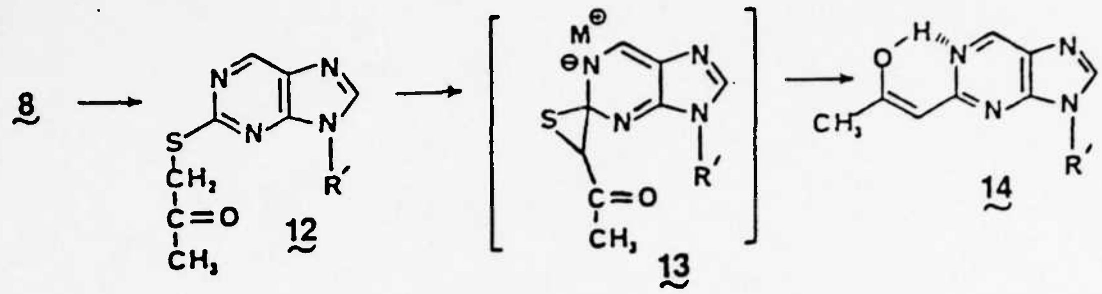
The synthesis of compound **1** commenced with guanosine (5) which was converted almost quantitatively (93%) in the first step to 2',3',5'-tri-O-acetylguanosine (6) by treatment with acetic anhydride, dimethylaminopyridine, and triethylamine in acetonitrile as solvent. The protected nucleoside **6**, when treated with phosphorus oxychloride and N,N-diethylaniline, was converted to the 2-amino-6-chloropurine nucleoside **7** in about 89% yield. Photolysis of **7** in dry, nitrogen-purged tetrahydrofuran (THF) containing 10% triethylamine produced the 2-amino nucleoside **8** in about 80% yield. This photoinduced reductive dehalogenation has not been reported previously in purine nucleoside chemistry and represents an excellent procedure for the synthesis of 2-aminopurines. Nucleoside **8** was designed as a key precursor for the synthesis of **1** and **3**.

Several approaches were examined for the synthesis of **1** from **8**. The first involved conversion of **8** to its 2-iodinated derivative with subsequent photoinduced $S_{RN}1$ reaction of this iodo compound with the potassium enolate of acetone (Scheme 1). Nucleoside **9** (i.e. the silylated derivative of **8**) was converted to the new 2-iodo-9-(2,3,5-tri-O-t-butyldimethylsilyl-D-ribofuranosyl)purine **10** in 67% yield by a deamination-halogenation reaction using n-pentyl nitrite, diiodomethane, and trimethylsilyl iodide in hexane. However, when **10** was photolyzed in the presence of the potassium enolate of acetone in THF at -48 °C for 20 minutes, the expected $S_{RN}1$ product was not isolated. Careful analysis of the high-field NMR, FTIR, UV, and mass spectral data suggested that nucleophilic attack with subsequent ring opening had occurred at the 6-position to produce **11**. Although this was totally unexpected, failure of the $S_{RN}1$ reaction may be attributed to a marked change in the reduction potential of **10** compared to the corresponding 6-iodo compound. The latter undergoes the $S_{RN}1$ reaction in very good yields. When the 6-position was blocked, as in the case of 2-iodo-6-methoxypurine nucleoside (a precursor for target molecule 2), the reaction still failed.



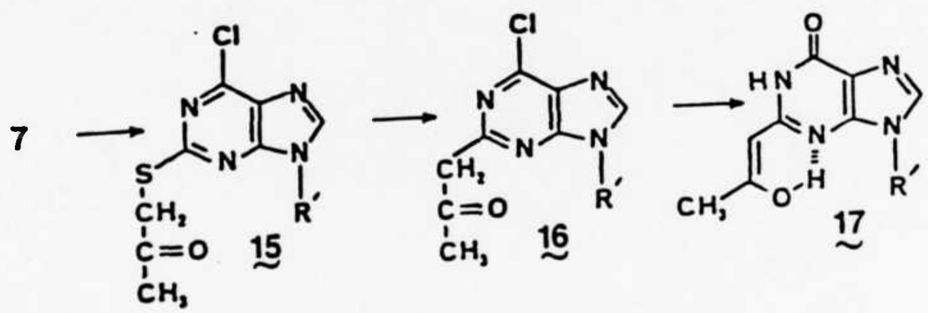
Scheme 1

An alternative approach to compound **1** also involved the use of the 2-amino nucleoside **8** as a precursor. The methodology involved conversion of **8** to its 2-thioacetonyl derivative and subsequent application of the Eschenmoser sulfide contraction on this thio derivative (Scheme 2). Nucleoside 8 can be converted to its 2-thioacetonyl derivative 12 in good yields by heating with n-pentyl nitrite and diacetonyl disulfide in acetonitrile. The Eschenmoser sulfide contraction reaction on 12 to give 14 <u>via</u> the intermediacy of 13 did not proceed as planned under a variety of conditions. Modifications in the experimental procedure included changing the solvent, the base, and the phosphine used.



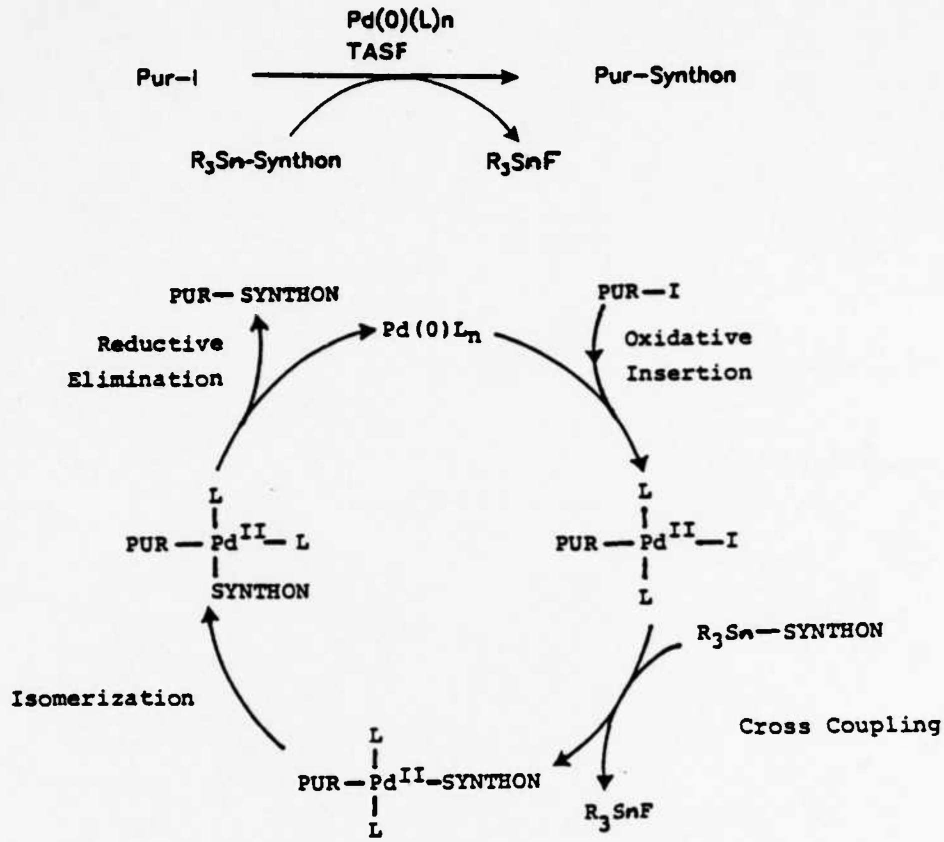
Scheme 2

A methodology involving radical addition of thioacetone at the 2-position of an appropriate precursor followed by thermal sulfide contraction and subsequent modification at the 6-position appeared also to be a promising and direct approach to the synthesis of **2** (Scheme 3). However, the sulfide contraction was also unsuccessful in this case. Application of the Meerwein reaction and radical coupling reactions did not provide suitable routes to **1** and **2**.



Scheme 3

Palladium is known to be able to insert into the carbon-iodine bond of an iodoaromatic system and this intermediate can subsequently undergo cross-coupling reactions with alkenes under suitable conditions. We have been very successful in developing this methodological approach for the synthesis of a number of the target nucleosides for this contract. The conceptual approach and a mechanistic explanation of the reaction is shown in Scheme 4. This conversion involves insertion of palladium into the carbon-iodine bond of the iodopurine followed by coupling of the derived Pd(II) complex with the tin enolate of acetone, trans-cis isomerization, and reductive elimination to give the product with regeneration of the Pd(0). Only catalytic amounts of palladium are required for this reaction. It is the first example of the use of an Sn reagent in palladium-catalyzed coupling involving nucleosides.

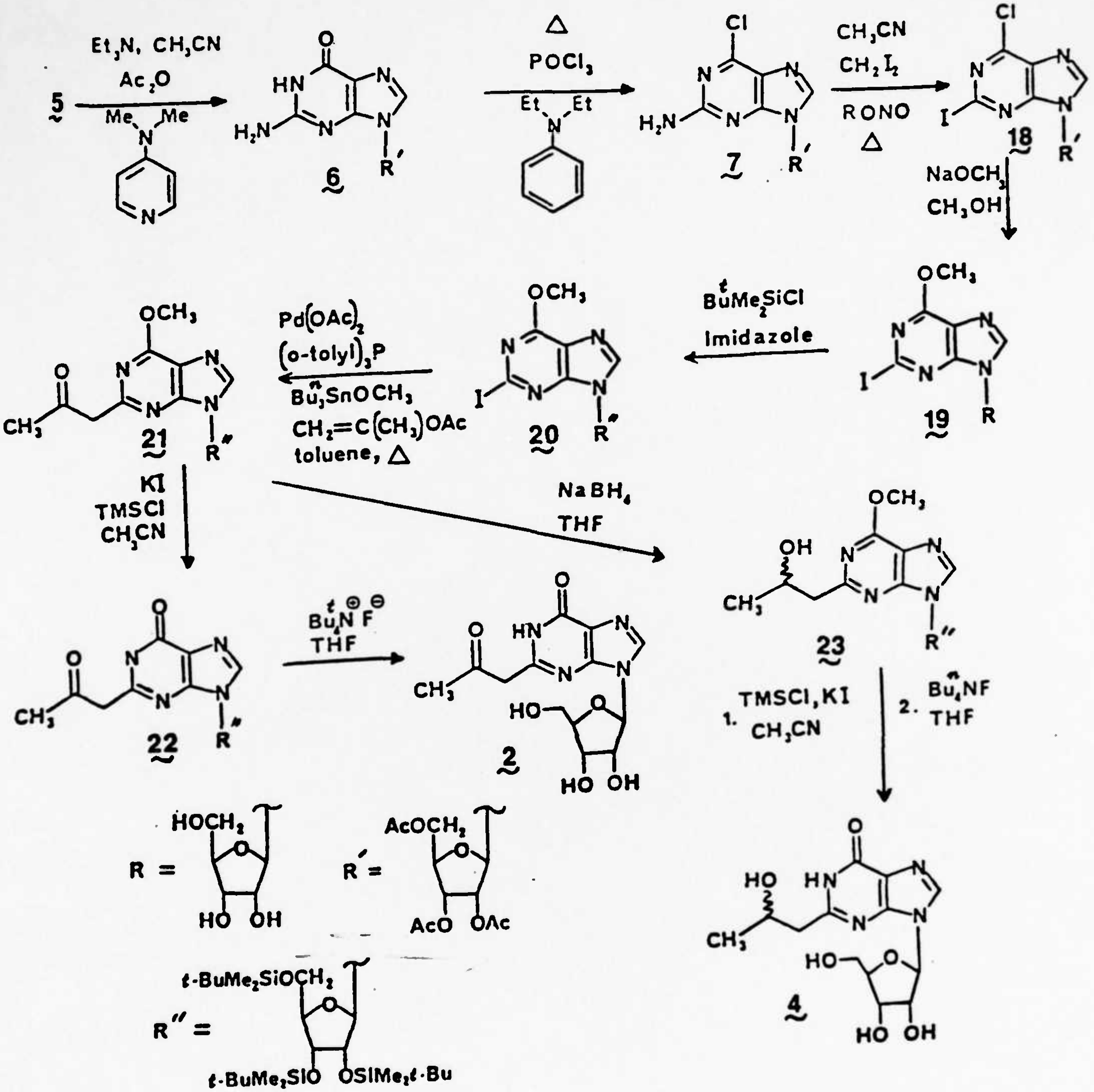


PUR—I = Iodopurine Nucleoside System

Scheme 4

The methodology is illustrated with the synthesis of **2** (Scheme 5). The synthesis commenced with guanosine (**5**) which was converted to the 2-amino-6-chloropurine nucleoside **7** in excellent yield as previously described in this report. Reaction of compound **7** with n-pentyl nitrite and diiodomethane in refluxing acetonitrile gave the 6-chloro-2-iodopurine nucleoside **18** in 71% yield. Replacement of the chlorine group at the 6-position with methoxide is accompanied by the desired deprotection of the acetate groups to give **19** in 76% yield. Subsequent protection of the carbohydrate moiety with t-butyldimethylsilyl chloride and imidazole in dimethylformamide gave **20** in 96% yield. The key step in the synthesis of **1** was the conversion of **20** to **21** in 70% yield by the palladium catalyzed coupling reaction discussed above. The acetonylated nucleoside **21** was converted to **2** in two steps by reaction first with trimethylsilyl iodide (64% yield of **22**) and subsequently with tetrabutylammonium fluoride (93% yield). The overall yield of **2** starting from guanosine was an excellent 17.9%.

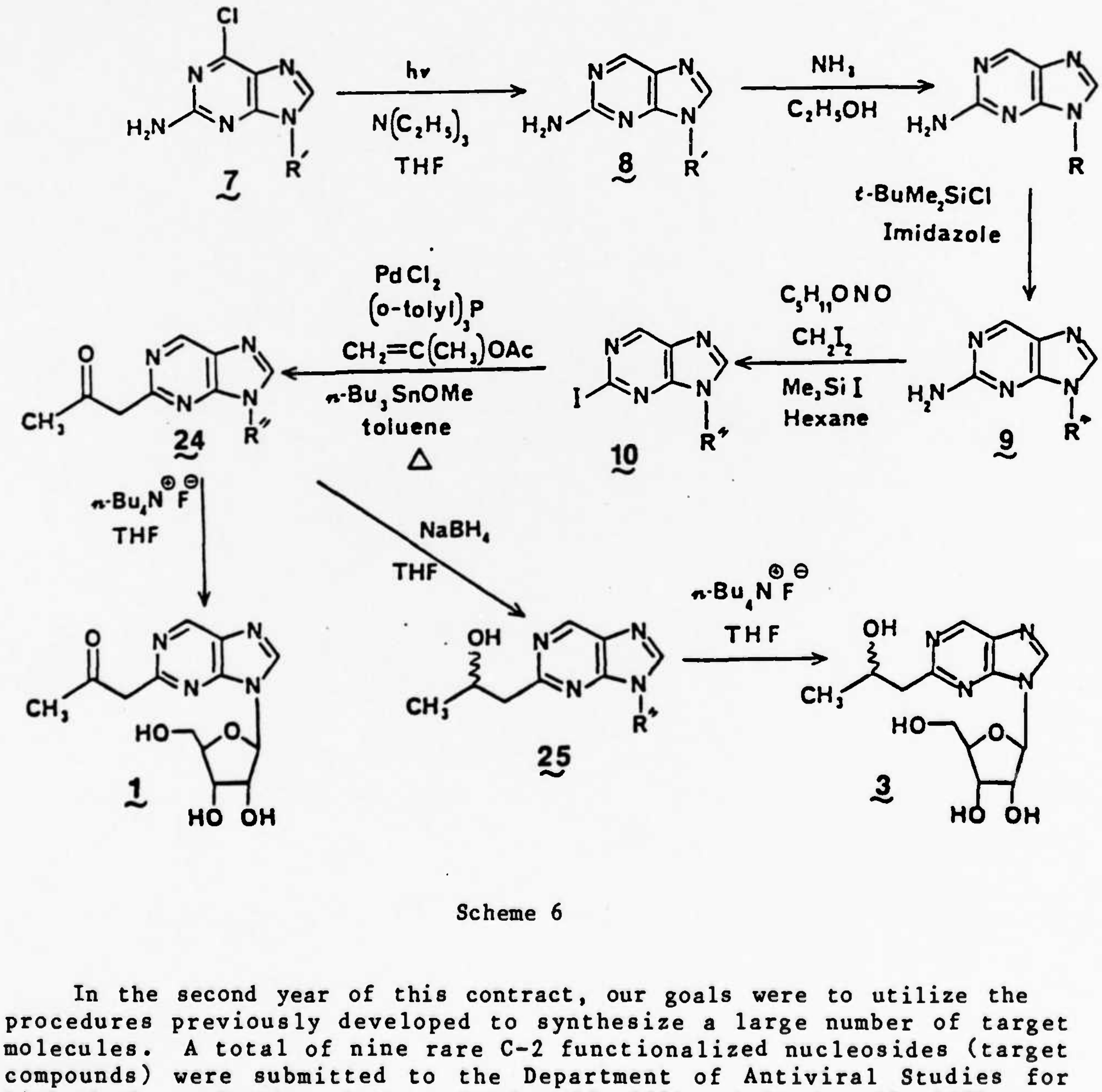
Compound **2** was purified to a high degree by reversed-phase high performance liquid chromatography (3 passes) on Amberlite XAD-4 resin using water-ethanol as the eluting solvent. The product may be crystallized from a water-isopropanol mixture. Complete characterization was performed by mass spectrometry, UV, FTIR, and high-field ^{1}H and ^{13}C NMR spectroscopy. Critical spectral data were presented with the sample submitted for antiviral testing.



Scheme 5

The starting material for the synthesis of compound 4 was the acetonylated nucleoside 21 (Scheme 5). It was smoothly reduced with sodium borohydride in tetrahydrofuran to give the diastereoisomeric products 23 in 75% purified yield. Compound 23 was deprotected to 4 in two steps by reaction first with trimethylsilyl iodide and subsequently with tetrabutylammonium fluoride. The overall yield of 4 starting from guanosine was an excellent 13%. Compound 4 was purified by multiple reversed phase HPLC on Amberlite XAD-4 with 5% ethanol-water as the eluting solvent. Crystallization may be carried out from isopropanol. Complete characterization was carried out by mass spectrometry, UV, FTIR, and high-field ^{1}H and ^{13}C NMR spectroscopy. Complete spectral data were provided with WRAMC FORM 108.

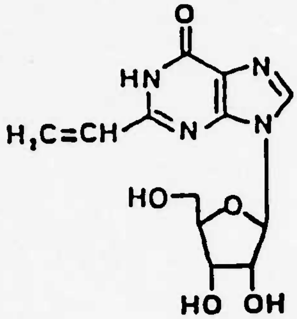
Using the aforementioned methodology, the preparations of 2-acetonyl-9-(β-D-ribofuranosyl)purine 1 and 2-(2-hydroxypropyl)-9-(β-D-ribofuranosyl)purine 3 were achieved. The approaches used for these compounds are outlined in Scheme 6. The precursor material for the syntheses was **10**, prepared from 7 as described previously (Scheme 1). The palladium-catalyzed reaction of **10** with the tributyltin enolate of acetone gave **24** in good yields. Compound **24** can be easily deprotected to **1** with tetrabutylammonium fluoride. It can be reduced with sodium borohydride to **25** which can be deprotected to **3**. Both compounds **1** and **3** were fully characterized and submitted for antiviral evaluation.



Scheme 6

In the second year of this contract, our goals were to utilize the procedures previously developed to synthesize a large number of target molecules. A total of nine rare C-2 functionalized nucleosides (target compounds) were submitted to the Department of Antiviral Studies for biological evaluation between October 18, 1986 and October 17, 1987.

The starting point of our work during the second year of the contract was the synthesis of the 2-vinyl compounds **26** and **27**. The rationale for the choice of these compounds as the starting point was that, in addition to being target molecules, they would also be key precursors for the synthesis of a variety of rare functionalized alkylated purine nucleosides.



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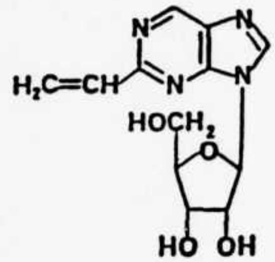
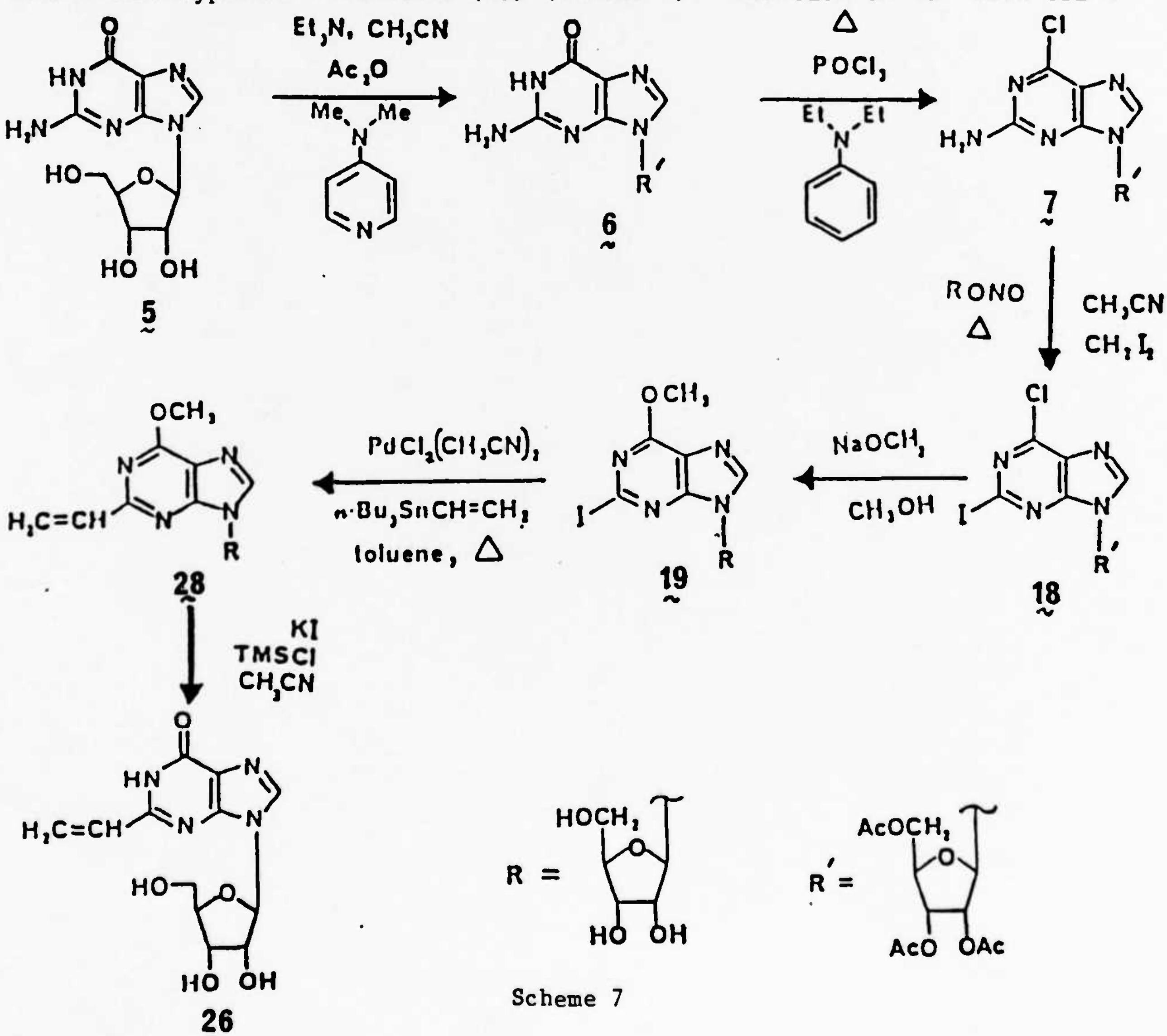


27

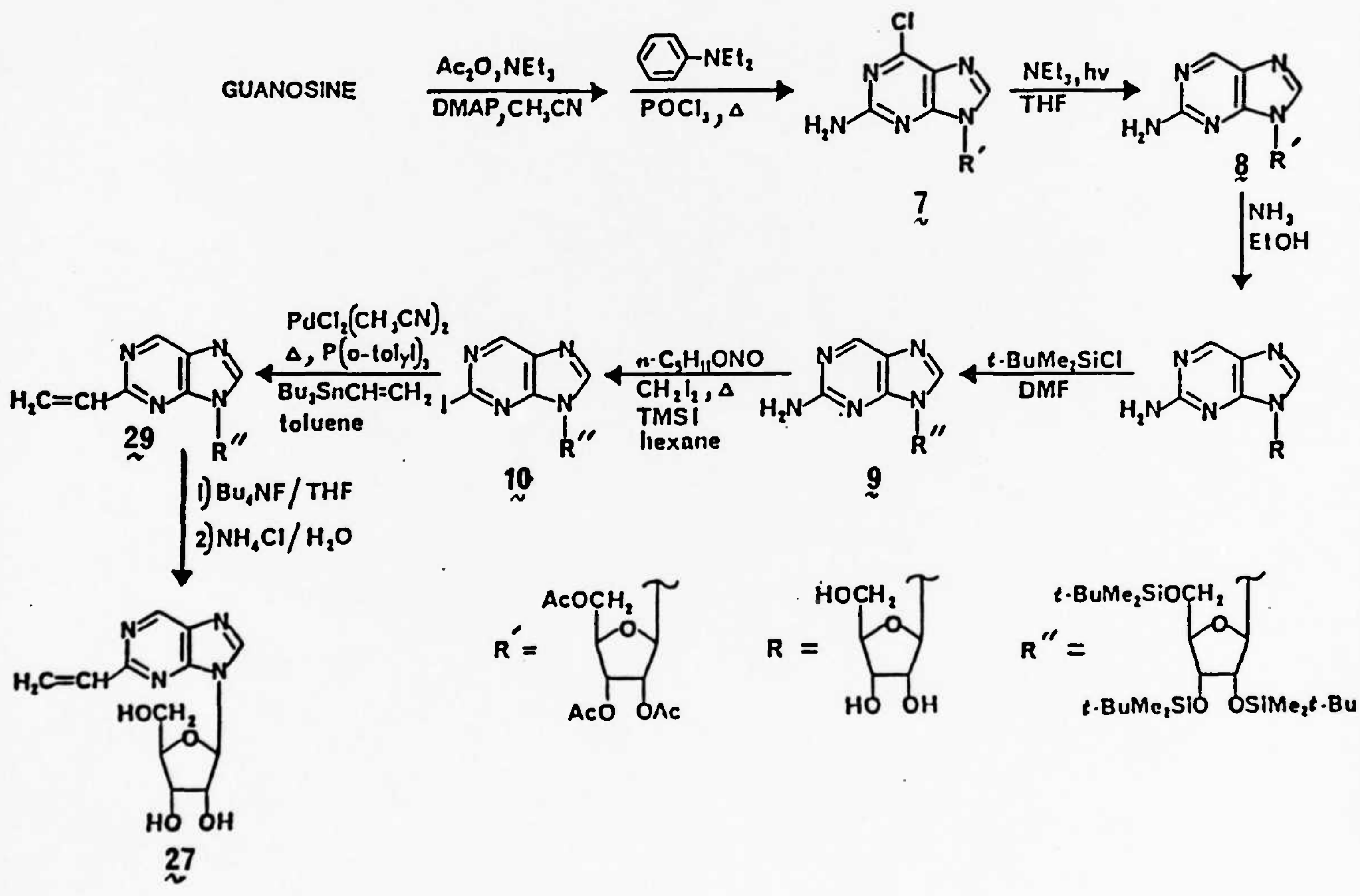
The synthesis of 2-vinylinosine (**26**) commenced with guanosine (**5**) which was converted in several steps, as described previously in the report, to 2-iodo-6-methoxypurine nucleoside (**19**) (Scheme 7). Reaction of **19** with tri-n-



Scheme 7

butylvinylstannane in the presence of palladium chloride gave **28** in > 90% yield. It should be noted that the palladium-catalyzed cross-coupling reaction was carried out on nucleoside **19** where the carbohydrate moiety was totally unprotected. Deprotection of **28** with trimethylsilyl iodide in acetonitrile resulted in cleavage of the methyl group to give the target molecule **26** in about 50 % yield after appropriate work up and purification. Our procedure for masking the hypoxanthine base in this way will find wide application in purine nucleoside chemistry. Compound **26** was purified by high performance liquid chromatography (three passes) on Amberlite XAD-4 resin with ethanol-water as the eluting solvent. Complete characterization was carried out by UV, FTIR, high-field ^{1}H and ^{13}C NMR spectroscopy, and elemental analysis.

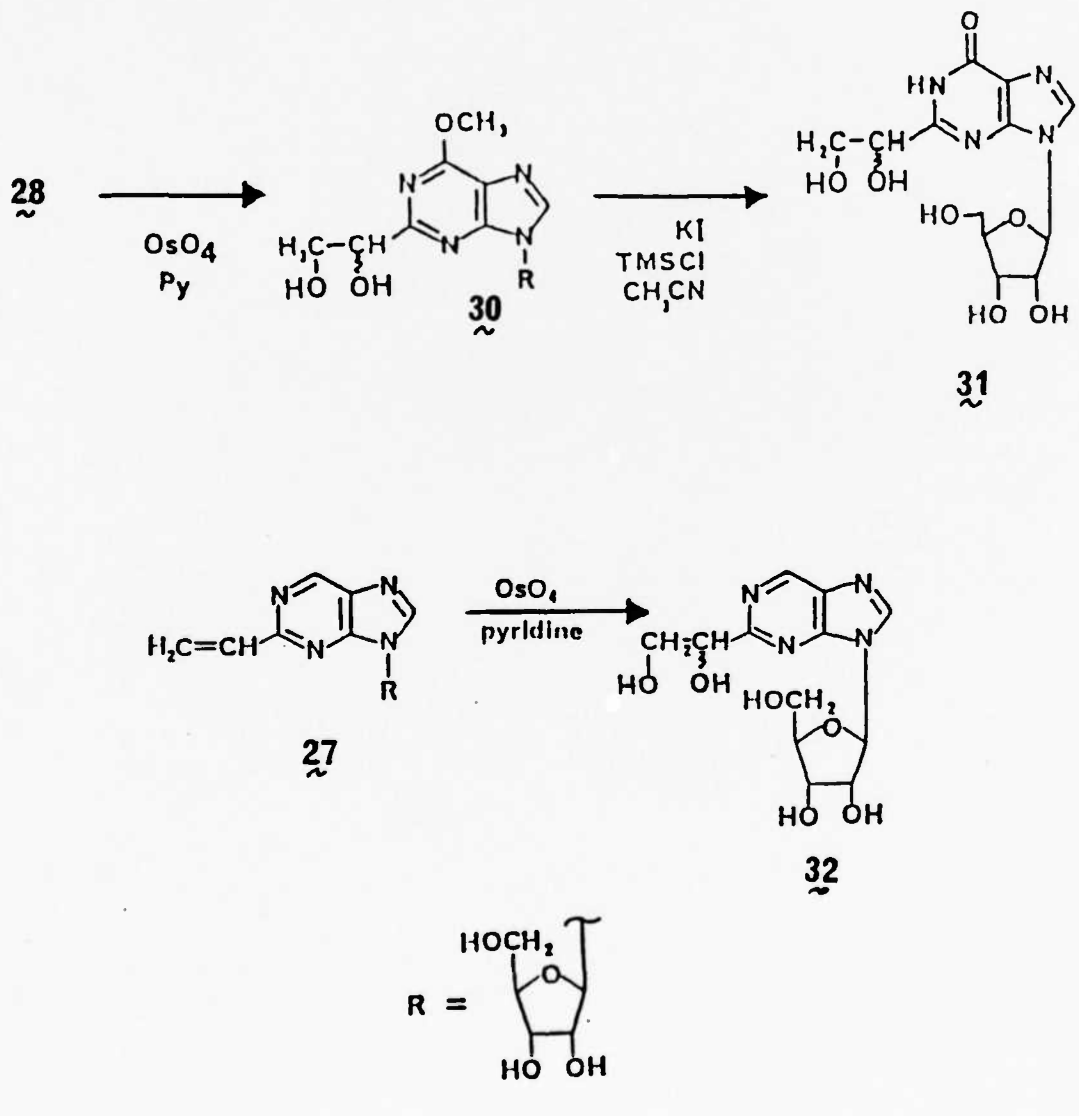
Compound **27** was synthesized using the sequence of reactions shown in Scheme 8. The starting material for the synthesis was the 6-chloropurine



Scheme 8

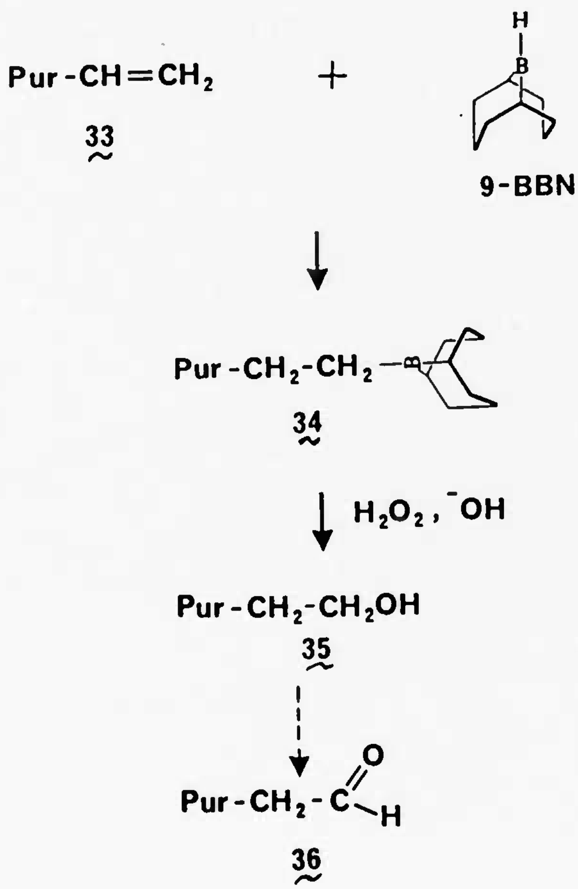
nucleoside **7** which was converted in several steps to the 2-iodopurine nucleoside **10** as shown in Scheme 8 and as described previously in this report. Reaction of **10** with tri-n-butylvinylstannane under palladium catalysis gave the 2-vinylpurine nucleoside **29** which was deprotected with tetrabutylammonium fluoride to the target molecule **27**. The overall yield starting from guanosine was 12%. The crude product was purified by flash chromatography followed by HPLC, fully characterized by spectral data, and submitted for antiviral evaluation.

Hydroxylation of compound **28** with osmium tetroxide gave the 1,2-dihydroxyethyl compound **30** in 55 % yield. Deprotection of **30** with trimethylsilyl iodide in acetonitrile gave **31** in 50 % yield (Scheme 9). This target molecule (obtained as a diastereoisomeric mixture) was purified by HPLC on Amberlite XAD-4 resin. It was fully characterized by spectral methods and elemental analysis. In a similar procedure, the vinylnebularine **27** was converted to the diastereoisomeric diols **32**.



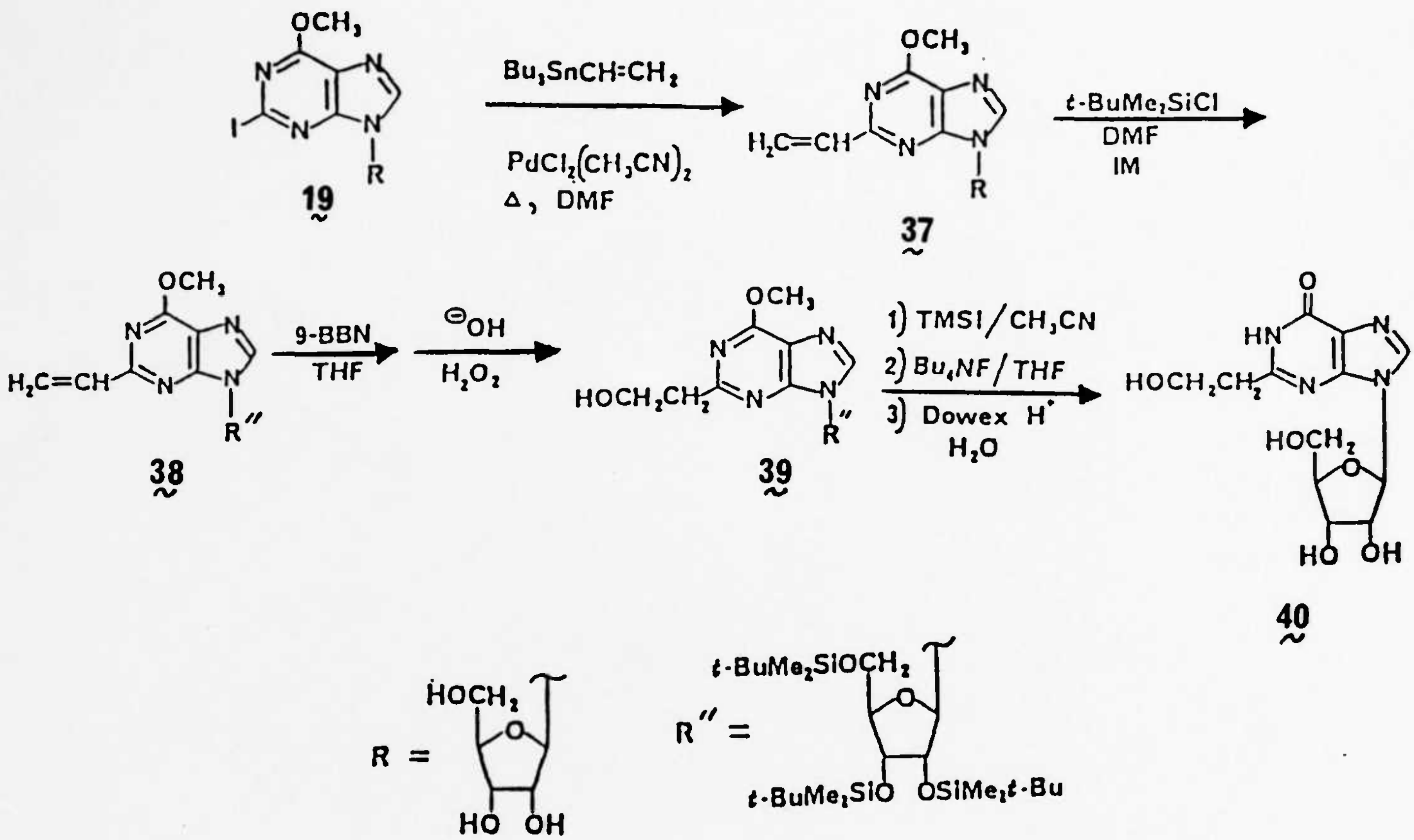
Scheme 9

The 2-vinyl purine nucleosides described could be elaborated further by selective oxidation at the beta-position to furnish other target molecules. The methodology for this conversion is outlined in Scheme 10. 9-Borabicylco [3.3.1]nonane (9-BBN) is expected to add regiospecifically to the vinyl group of **33** to give the borane **34** with the boron bearing moiety at the terminal position. Oxidation of **34** with alkaline hydrogen peroxide would furnish the desired alcohol **35**. It was planned that target molecules **35** would be precursors for the corresponding aldehydes **36** <u>via</u> controlled oxidation.
With respect to the hydroboration reaction, it should be mentioned that such reactions have rarely been used to elaborate structures in nucleoside chemistry.



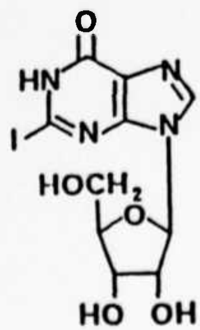
Scheme 10

Thus, the rare functionalized inosine analogue, 2-(2-hydroxyethyl)inosine **40**, was synthesized from the 2-vinyl compound **37**. This compound was first protected by silylation to give **38**, which was then treated with 9-borabicyclo [3.3.1]nonane (9-BBN). Oxidative work-up of the resulting organoborane gave the alcohol **39** in 52% yield. High-field ^{1}H NMR spectral data confirmed that the regiospecificity of reaction as well as the structure of the isomer isolated. Deprotection of **39** with trimethylsilyl iodide in acetonitrile followed by treatment with fluoride ions gave the target alcohol **40** (Scheme 11). Purification and characterization were carried out as described for other target compounds.



Scheme 11

In the last part of the second year of support, four compounds were submitted for antiviral evaluation. Two of the compounds, **41** and **42**, were prepared by deprotection of key halogenated intermediates used in the syntheses previously described in this report. The other two compounds were target ketones **44** and **46** in which special emphasis was placed because of the potent antiviral activity of another ketone, 2-acetonylinosine, previously synthesized by us in this program.



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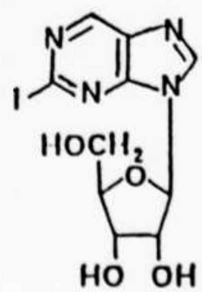
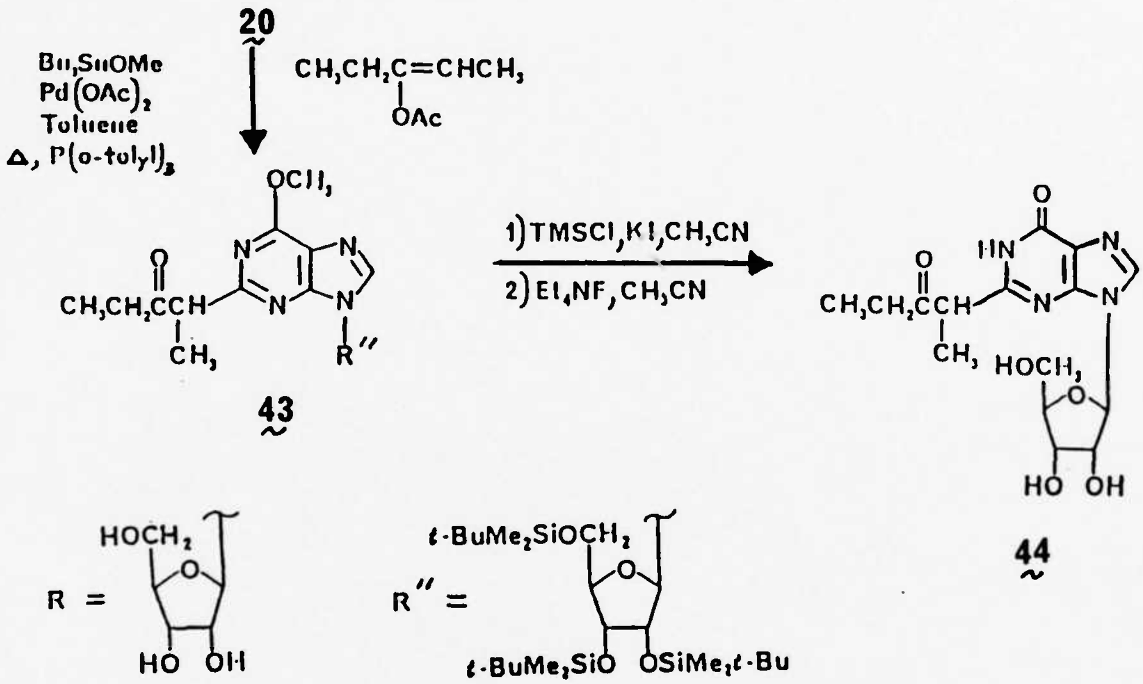


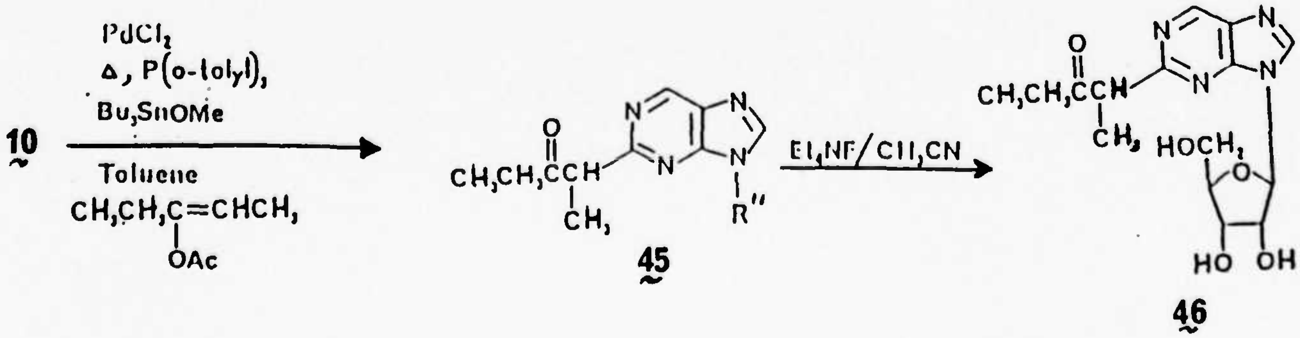
42

The immediate precursor for the synthesis of the 2-ketoinosine **44** was the silylated 2-iodo compound **20**. When compound **20** was heated under reflux in toluene with palladium acetate, tri-o-tolylphosphine, tri-n-butyltin methoxide, and 2-pentene-3-acetate, very good yields of the keto compound **43** was obtained. The latter was deprotected to the target molecule **44** in two steps, first by reaction with trimethylsilyl iodide and then with tetraethylammonium fluoride (Scheme 12). Target compound **44** was purified by reversed-phase HPLC. The overall yield of **44** starting from guanosine was 10.8 %. It was fully characterized by spectral methods and by high-resolution fast atom bombardment mass spectrometry (FAB HRMS).



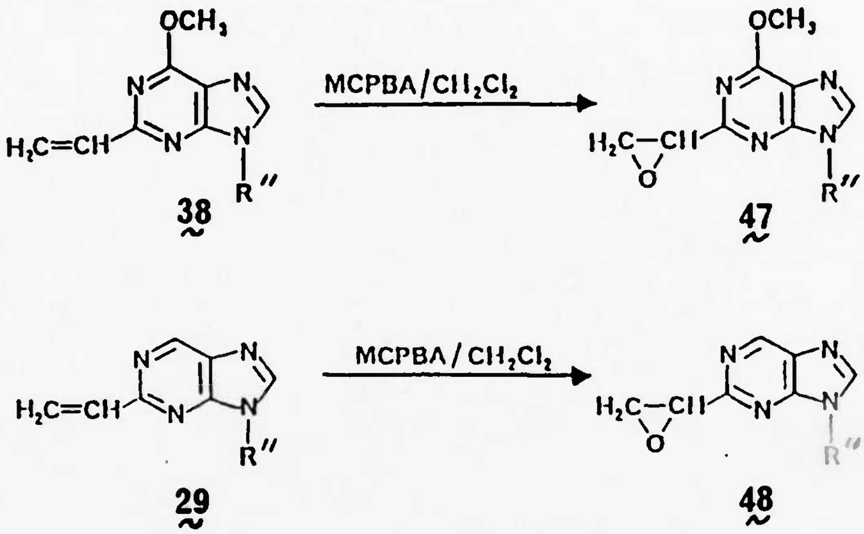
Scheme 12

Synthesis of the ketonebularine **46** was achieved using the silylated 2-iodopurine nucleoside **10** as the immediate precursor. The palladium-catalyzed cross-coupling reaction of **10** to give **45** was carried out as described above for the conversion of **20** to **43**. Excellent yields of product were obtained in this conversion. Deprotection of **45** (tetraethylammonium fluoride) followed by purification of the resulting material by HPLC, gave target molecule **46** (13.7% overall yield from guanosine).



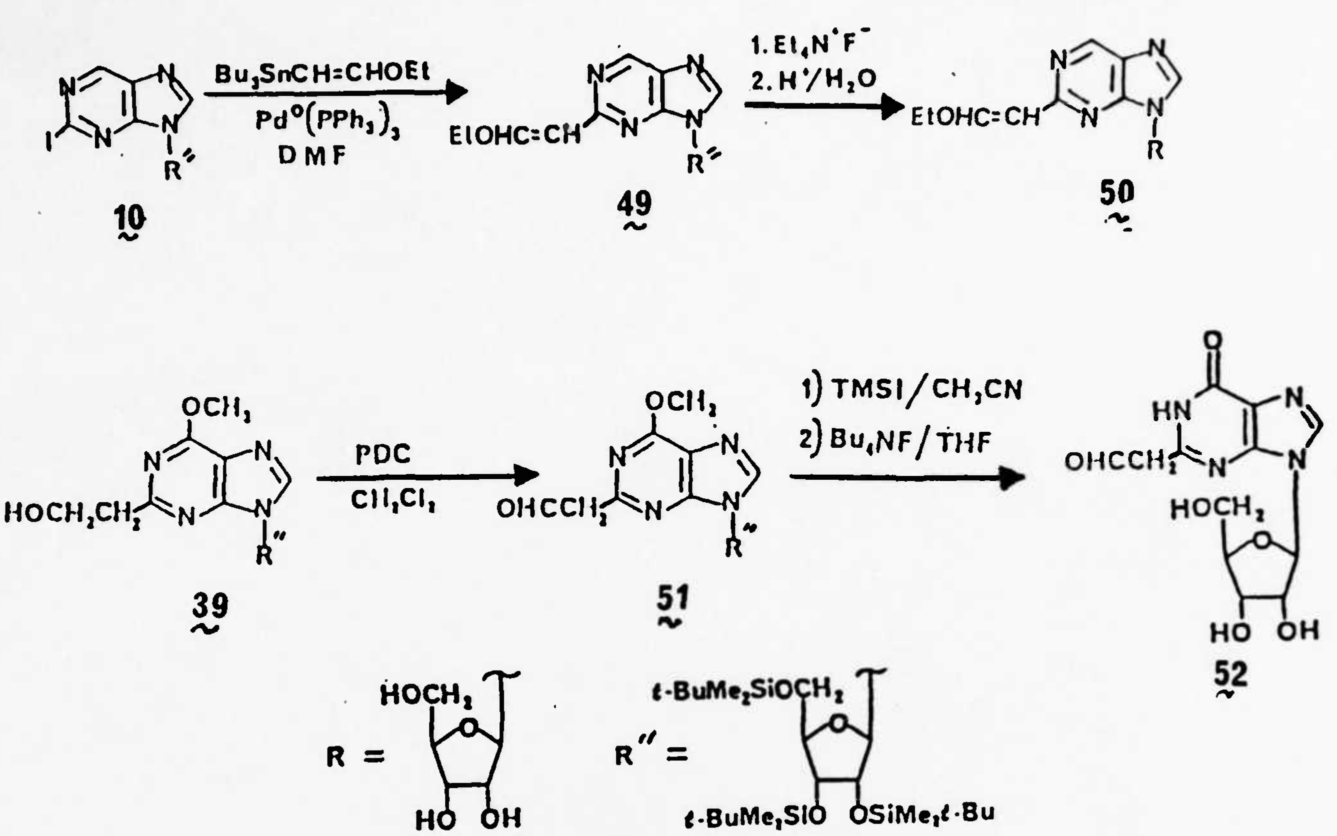
Scheme 13

Epoxy substituted purine nucleosides are very rare compounds and only one example of a purine system with an epoxy group at the 6-position is known (Nair and Chamberlain, J. Am. Chem. Soc. 1985, 107, 2183). The approach to the 2-epoxy compounds of the inosine and nebularine series was through the corresponding vinyl compound precursors (38 and 29) whose synthesis have been described previously in this report. Although epoxidation of these vinyl compounds appeared to have proceeded as expected to give the epoxides 47 and 48 (Scheme 14), isolation of the epoxide products was extremely difficult because of their inherent instability. Several different procedures for isolation and deprotection were attempted, but all were unsuccessful.



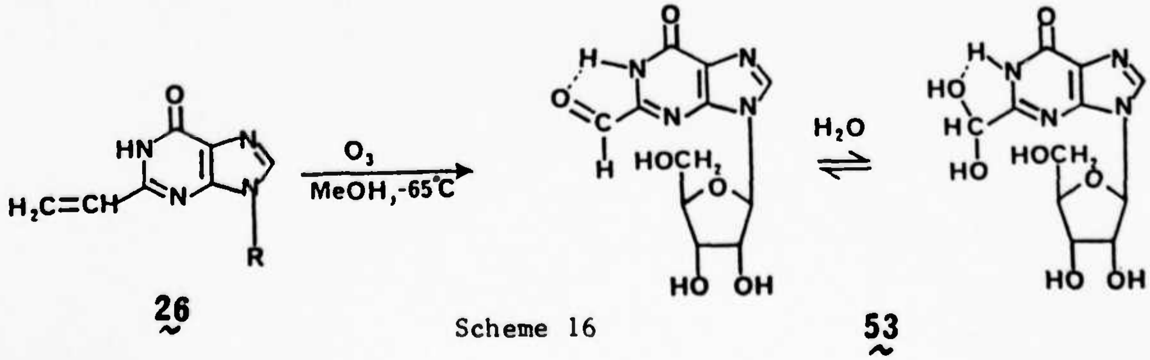
Scheme 14

During the second year of the contract, we were also involved in the development of approaches to the synthesis of analogues of nebularine and inosine that contain aldehyde functionalized carbon-carbon bonding at the 2-position. The initial approach for the nebularine series was to synthesize the 2-(2-hydroxyethyl)purine system and selectively oxidize this primary hydroxyl group to the aldehyde. Although the synthetic procedure for the preparation of the 2-hydroxyethyl derivative of inosine had previously been developed by us, application of this to the nebularine series (i.e. hydroboration followed by oxidative work-up) resulted in the formation of the 2-ethyl compound through reduction of the intermediate organoborane. An alternative procedure involved direct introduction of a masked aldehyde moiety at the C-2 position. This was achieved through the use of ethyl vinyltributyltin ether. This organostannane was prepared by the radical coupling of tributyltin hydride with ethyl ethynyl ether. Palladium-catalyzed coupling of the organostannane with protected 2-iodopurine nucleoside 10 gave the (E)- and (Z)- mixture of the expected product 49 in about 70% yield (Scheme 15). However, although removal of the silyl protecting groups from 49 could be achieved to give 50, attempted unmasking of vinyl ether group gave an intractable mixture under a variety of conditions. The same problem was encountered when deprotection of compound 51, prepared from 39 by PDC oxidation, was attempted (Scheme 15).



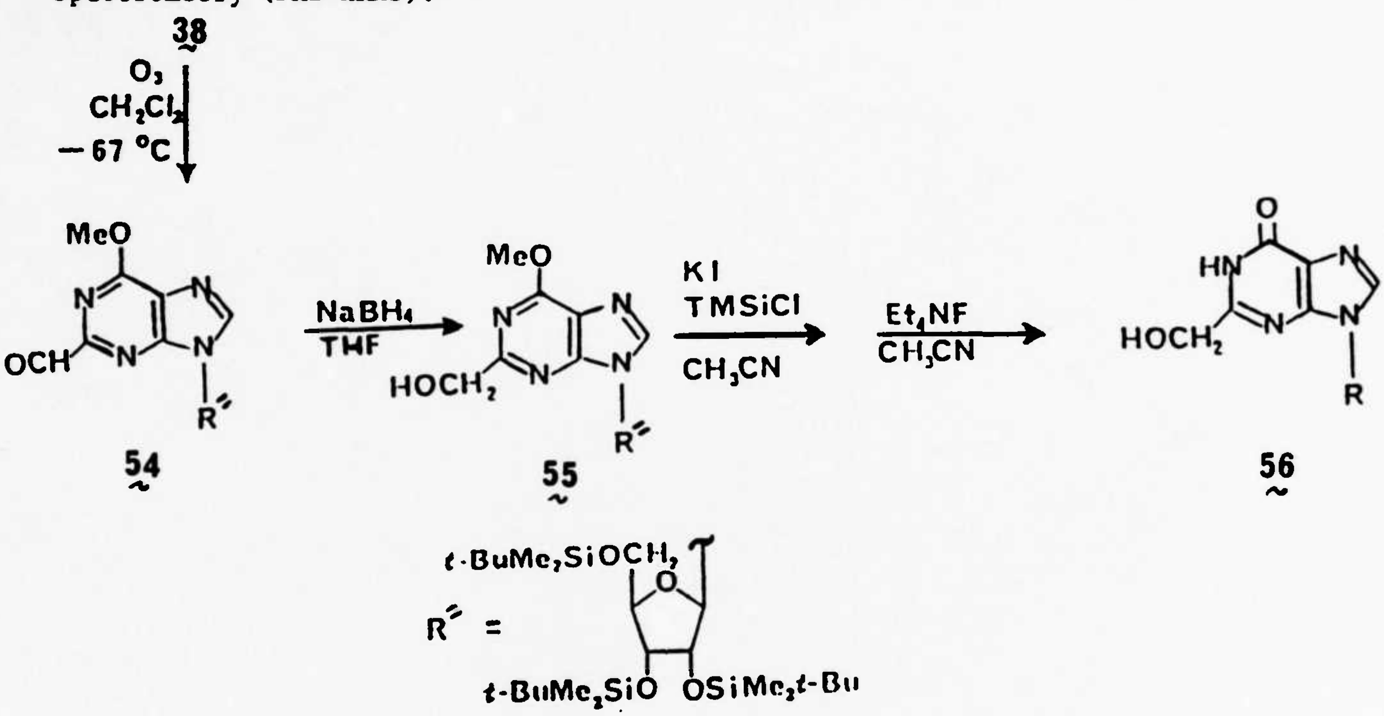
Scheme 15

The starting point of our work during the third and final year of this contract was the synthesis of 2-formylinosine 53. The immediate precursor for the synthesis of this rare nucleoside was 2-vinylinosine 26 prepared as previously described from the reaction of the 2-iodo compound 19 with vinyltributyltin under palladium catalysis, followed by deprotection with trimethylsilyl iodide (Scheme 16). When 2-vinylinosine was subjected to ozonolysis under carefully controlled conditions and the reaction mixture reductively worked up, 2-formylinosine (53), was isolated in about 50% yield. This target compound was purified by reversed-phase HPLC on Amberlite XAD-4 resin with water-ethanol as the eluting solvent. The overall yield of 53 from guanosine was 14 %. This compound was fully characterized by UV, FTIR, high-field ^{1}H and ^{13}C NMR, and high resolution fast atom bombardment mass spectrometry (FAB HRMS). The compound exists in both the formyl and its hydrated form and evidence for the presence of both forms can be easily discerned from the carbon spectrum. The chemical shift of the aldehyde carbon at about 185 ppm is consistent with the amide-like character of this carbonyl group.



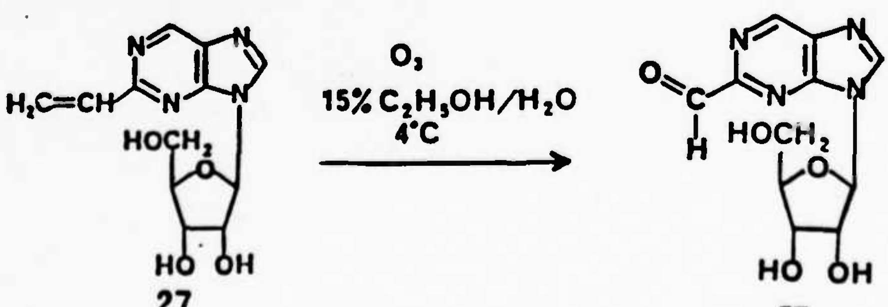
Scheme 16

The key precursor for the synthesis of 2-hydroxymethylinosine (56) was the 2-vinyl compound 38 (Scheme 17). Controlled ozonolysis of compound 38 followed by reductive work-up with dimethyl sulfide gave the 2-formylinosine 54 in 54% yield. Reduction of 54 with sodium borohydride gave the alcohol 55 in 96% yield. Deprotection of 55 with trimethylsilyl iodide followed by tetraethylammonium fluoride gave the target compound 56. Target molecule 56 was purified by reversed-phase HPLC on Amberlite XAD-4 resin with water-ethanol as the eluting solvent. The overall yield of 56 starting from guanosine was about 18%. This compound was characterized by UV, FTIR, high-field ^1H and ^{13}C NMR, and high-resolution fast atom bombardment mass spectrometry (FAB HRMS).



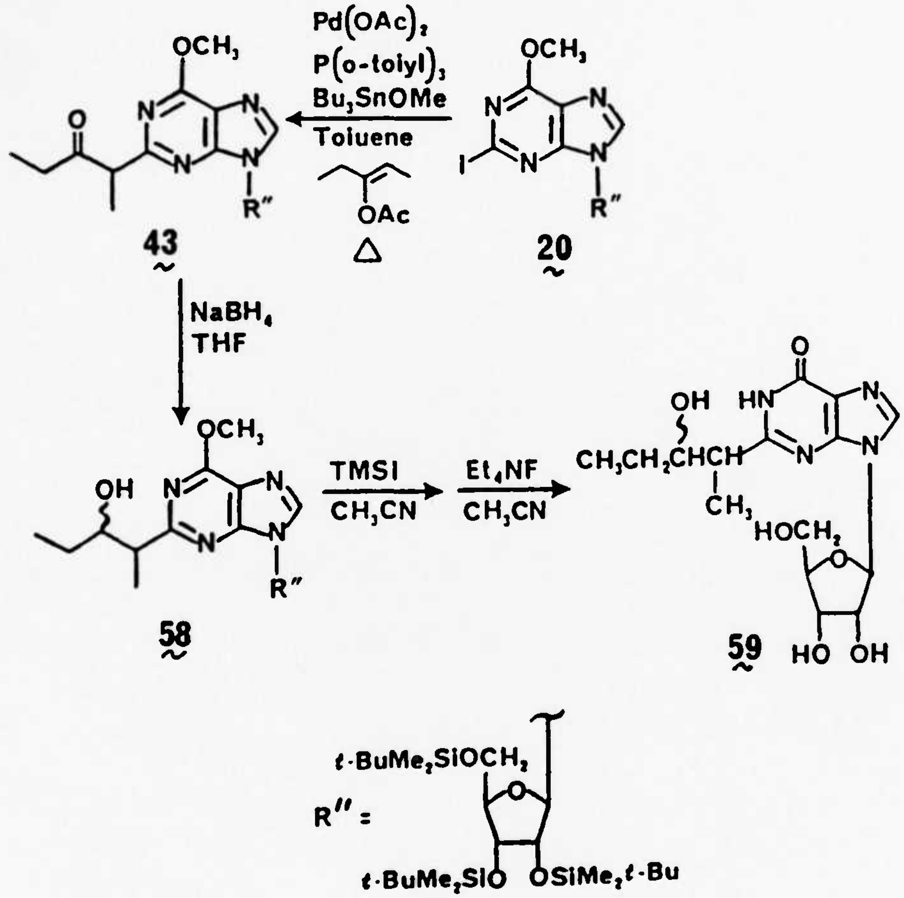
Scheme 17

2-Vinylnebularine (27) is the immediate precursor for the synthesis of the novel nebularine analogue, 2-formylnebularine (57). Ozonolysis of 27 under carefully controlled conditions followed by reductive work up gave 2-formylnebularine (57) in 61% yield (Scheme 18). This target molecule was purified by reversed-phase HPLC and fully characterized. The overall yield of 57 starting from guanosine was 11 %.



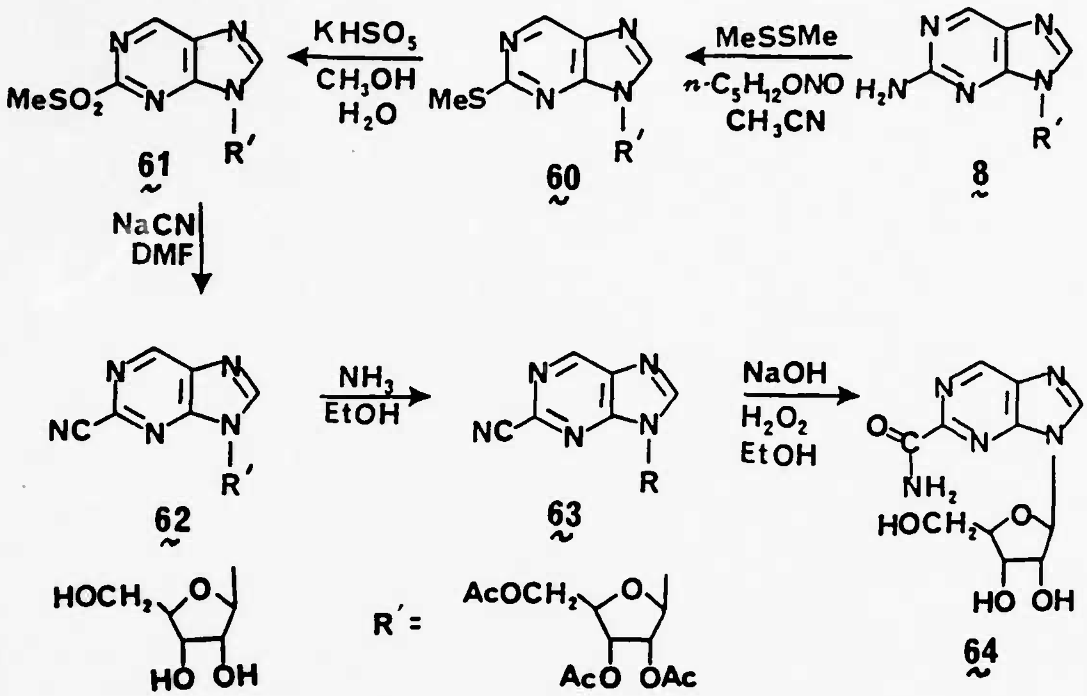
Scheme 18

The immediate precursor for the synthesis of the C-2 functionalized alcohol **59**, also submitted for antiviral evaluation during the third year, is the 2-keto purine ribonucleoside **43**, prepared from silylated 2-iodo-6-methoxypurine ribonucleoside **20** by a palladium-catalyzed cross-coupling reaction with tributyltin methoxide and 2-pentene-3-acetate. Reduction of **43** with sodium borohydride gave the diastereoisomeric alcohols **58** in 77% yield. Deprotection of **58** with trimethylsilyl iodide followed by tetraethylammonium fluoride and purification of the resulting product by HPLC gave the target molecule **59** (Scheme 19, 11% overall yield from guanosine).



Scheme 19

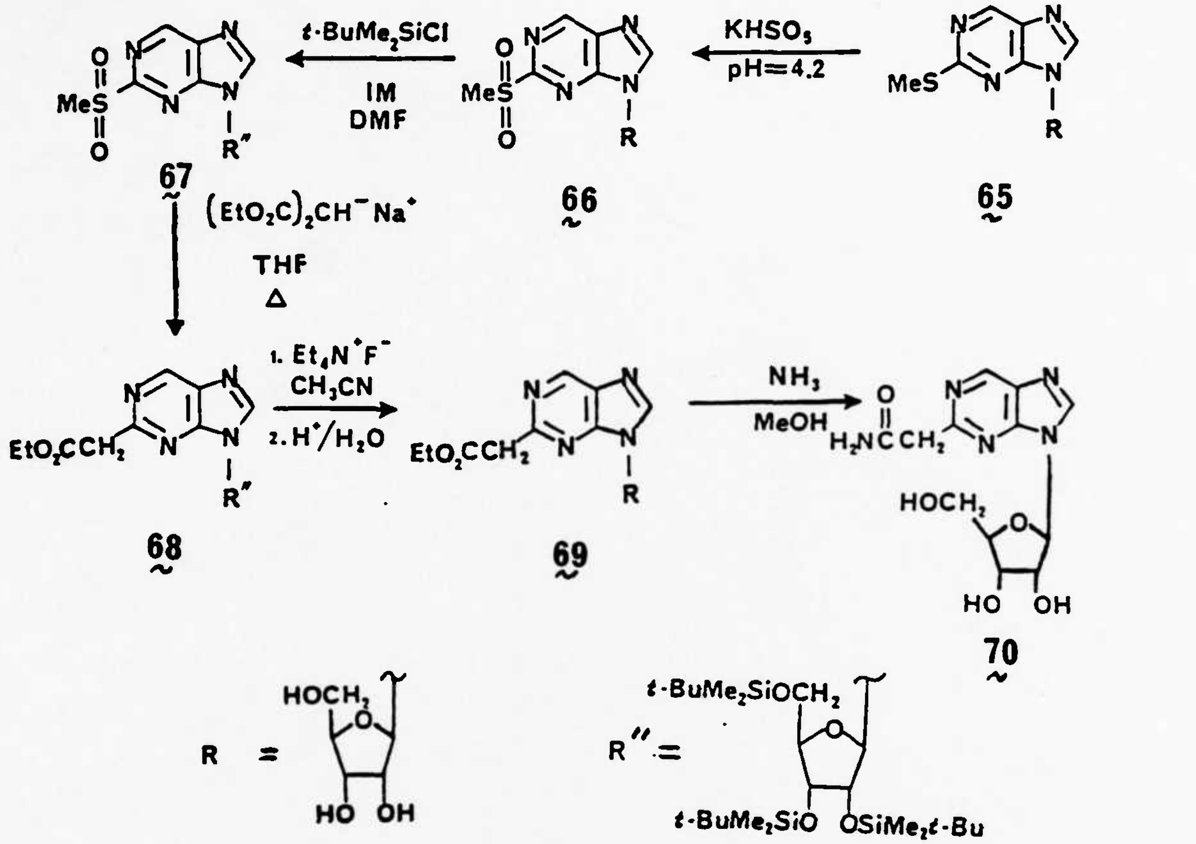
In the third year, we were also successful in synthesizing the 2-carboxamidonebularine **64**. This compound was synthesized by a methodology different from that used for the previous two compounds. A key starting compound for this synthesis was **8**. When the 2-amino compound **8** was treated with n-pentyl nitrite and dimethyl disulfide in acetonitrile, thioalkylation occurred to give **60** as the product in 41% yield (Scheme 20). Oxidation of **60** with oxone proceeded smoothly to give the sulfoxide **61** in 75% yield. The $S_{RN}1$ reaction of **61** with cyanide ion followed by deprotection gave 2-cyanonebularine (**63**) in 30% overall yield. 2-Cyanonebularine is a new nucleoside. Hydrolysis of this compound (NaOH, H_2O_2, EtOH) gave the carboxamide **64** in 31% yield. The overall yield of this new nucleoside starting from guanosine was 1 %. It was purified, characterized, and submitted for antiviral evaluation.



Scheme 20

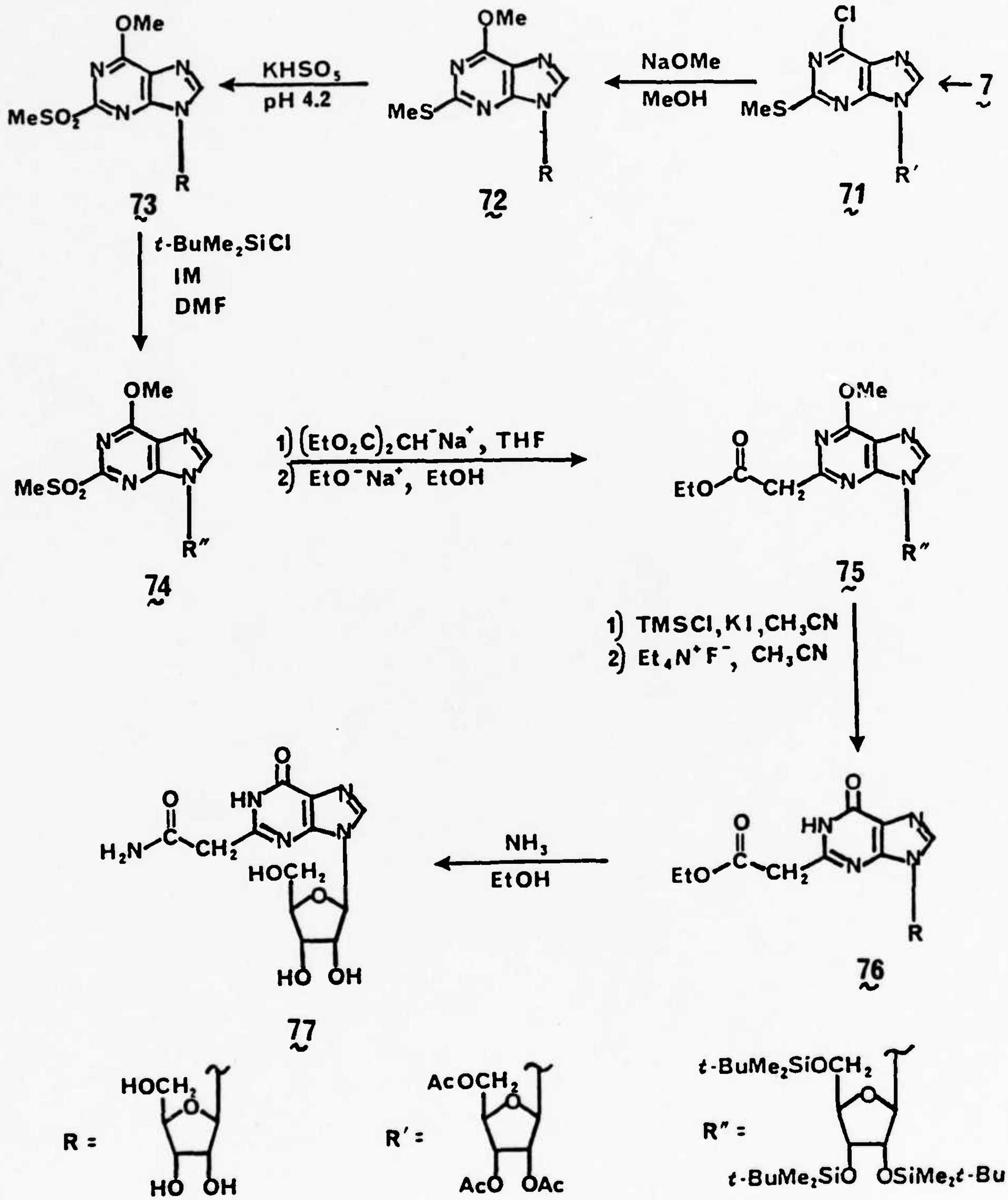
Synthesis of the novel carboxamide **70** was carried out starting with compound **60**. Deprotection of **60** with sodium methoxide in methanol and subsequent oxidation with oxone converted **60** in to the sulfone **66** (25% overall yield from **8**). Silylation of **66** (72%) followed by an $S_{RN}1$ reaction with the anion of diethyl malonate resulted in the formation of **68** (72%) (Scheme 21). It appears that **67** is initially converted to the diethyl malonyl derivative which undergoes a base-catalyzed retro-Claisen to **68**. Deprotection of **68** (81%) followed by reaction of the resulting compound (**69**) with methanolic ammonia gave the target amide **70** in 75% yield after purification by reversed-phase HPLC. Compound **70** was characterized by spectroscopic methods and submitted for antiviral evaluation.

Synthesis of the target molecule, 2-acetamidoinosine **77**, was first attempted via the base-catalyzed hydrolysis of the precursor nitrile, synthesized from the interesting palladium-catalyzed cross-coupling reaction between **20** and tri-n-butyl(cyanomethyl)stannane. However, this hydrolysis was unsuccessful under a variety of conditions. An alternative approach, however, was completely successful (Scheme 22). The starting compound for this approach was the 2-amino nucleoside **7**. A thermal radical deamination thioalkylation of **7** followed by deprotection with sodium methoxide in methanol and subsequent oxidation with oxone converted **7** in about 38% overall yield to the sulfone **73**. Silylation of **73** (63 %) followed by an $S_{RN}1$ reaction with the anion of diethyl malonate resulted in the formation of the 2-malonate of **74**. Reaction of the latter with sodium ethoxide in refluxing ethanol gave **75** in 60% yield (for the two steps). Deprotection of **75** (42%) followed by reaction of the resulting compound (**76**) with methanolic ammonia gave the target amide **77** in 50% yield after purification by reversed-phase HPLC. Compound **77** was characterized by UV, FTIR, FAB HRMS, and NMR data and submitted.

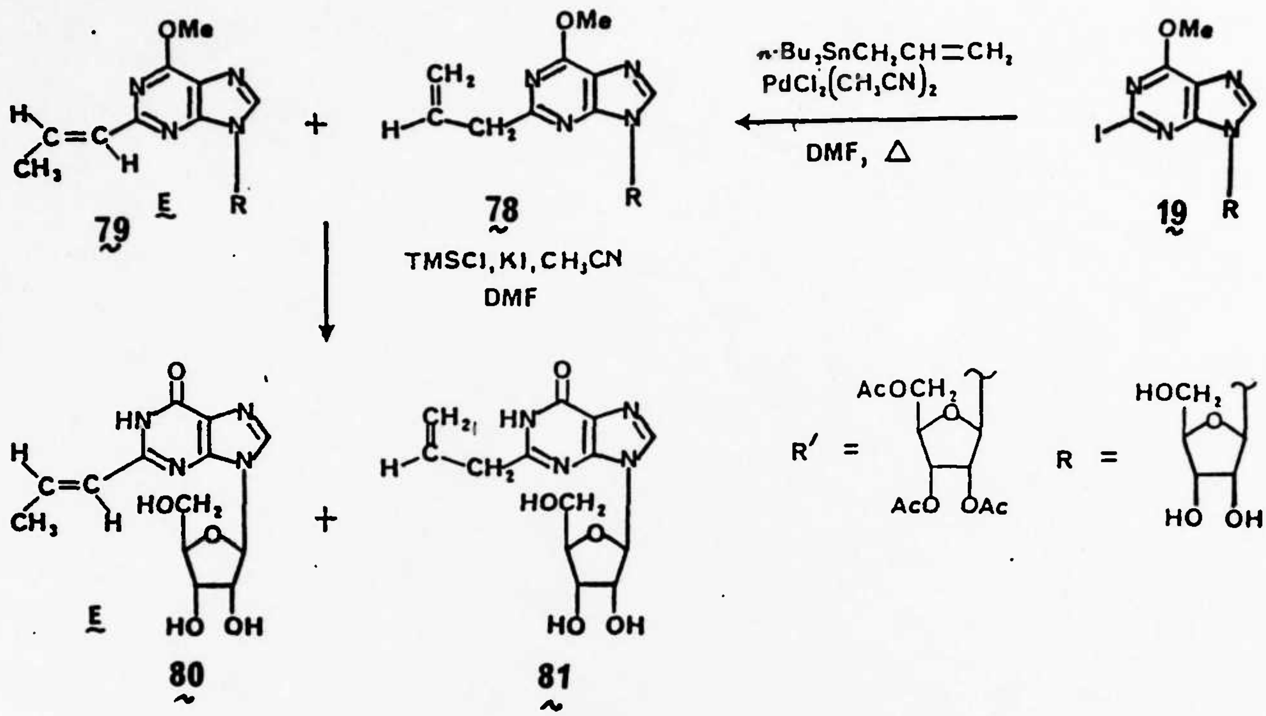


Scheme 21

The key precursor for the synthesis of E-2-(1-propenyl)inosine **80** was the 2-iodo-6-methoxypurine nucleoside **19** (Scheme 23). Treatment of **19** with bis(acetonitrile)palladium II chloride with allyl tri-n-butylstannane in DMF at 100 °C for 6h, afforded a mixture of the 2-allyl-6-methoxypurine [2-(2-propenyl)-6-methoxypurine] **78**, and the rearranged product, 2-(1-propenyl)-6-methoxypurine **79** in a ratio of 2.5 to 1 and a combined yield of 92%. It is likely that the isomerization of the 2-propenyl to the 1-propenyl group is occurring at the slow step of the reaction, i.e. the transmetalation stage. Temperature appears to be an important factor in these reactions. Below 90 °C, the reaction is extremely sluggish. The formation of the rearranged product is minimum at 90 °C. On the other hand, exclusive formation of the 1-propenyl compound **79** is realized when the temperature is raised to 110 °C. Deprotection of **79** with trimethylsilyl iodide in acetonitrile/DMF gave the target compound **80** in 81% yield after purification by reversed-phase HPLC on Amberlite XAD-4 resin. Complete structural characterization was carried out by UV, FTIR, FAB HRMS, and NMR data. The high-field ^1H NMR spectrum (in DMSO-d_6) gave unequivocal evidence for the E-stereochemistry of the exocyclic double bond (J=17.0 Hz).


OMe
KHSO5
pH 4.2
NaOMe
MeOH
Cl
MeSO2
MeS
R
R'
73
72
71
7
t-BuMe2SiCl
IM
DMF
1) (EtO2C)2CH⁻Na⁺, THF
2) EtO⁻Na⁺, EtOH
EtO
CH2
R"
74
75
1) TMSCl,KI,CH3CN
2) Et4N⁺F⁻, CH3CN
HN
H2N
HOCH2
HO OH
NH3
EtOH
76
77
R =
R' =
R" =
AcOCH2
AcO OAc
t-BuMe2SiOCH2
t-BuMe2SiO OSiMe2t-Bu

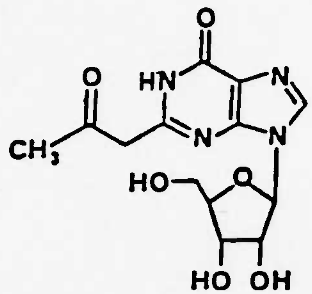
Scheme 22


OMe
n-Bu$_3$SnCH$_2$CH=CH$_2$
PdCl$_2$(CH$_3$CN)$_2$
DMF, Δ
79
E
78
19
R
TMSCl, KI, CH$_3$CN
DMF
80
81
R′ =
AcOCH$_2$
AcO OAc
R =
HOCH$_2$
HO OH

Scheme 23

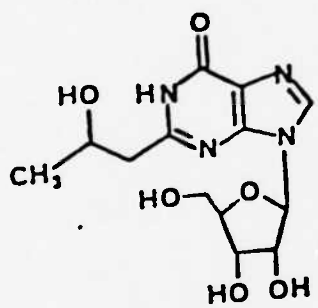
6. List of Target Compounds and Intermediates Submitted:

(i) 2-Acetonylinosine
or 2-Acetonyl-9-(β-D-ribofuranosyl)hypoxanthine



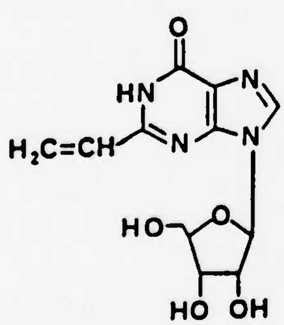
AVS Identifying Number: AVS-002159
Contractor's Identifying Code No: VN-I-101
Final Report Reference: Scheme 5

(ii). 2-(2-Hydroxypropyl)inosine
or 2-(2-Hydroxypropyl)-9-(β-D-ribofuranosyl)hypoxanthine



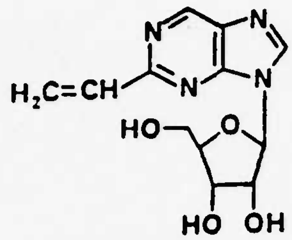
AVS Identifying Number: AVS-002352
Contractor's Identifying Code No: VN-I-102
Final Report Reference: Scheme 5

(iii) 2-Vinyl-9-(β-D-ribofuranosyl)hypoxanthine or 2-Vinylinosine



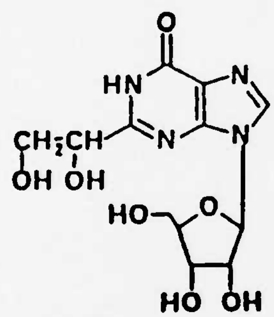
AVS Identifying No: AVS-002716
Contractor's Identifying Code No: VN-I-103
Final Report Reference: Scheme 7

(iv) 2-Vinyl-9-(β-D-ribofuranosyl)purine or 2-Vinylnebularine



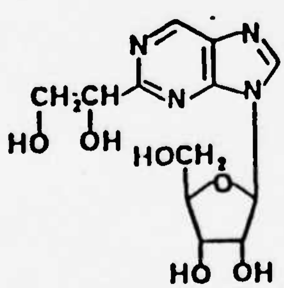
AVS Identifying No: AVS-002694
Contractor's Identifying Code No: VN-I-104
Final Report Reference: Scheme 8

(v) 2-(1,2-Dihydroxyethyl)-9-(β-D-ribofuranosyl)hypoxanthine or 2-(1,2-Dihydroxyethyl)inosine



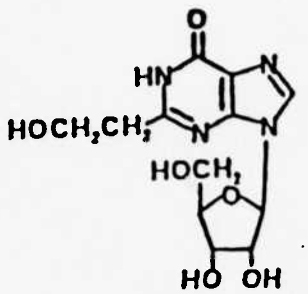
AVS Identifying No: AVS-002695
Contractor's Identifying Code No: VN-I-105
Final Report Reference: Scheme 9

(vi) 2-(1,2-Dihydroxyethyl)-9-(β-D-ribofuranosyl)purine or 2-(1,2-Dihydroxyethyl)nebularine



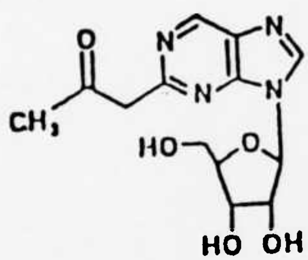
AVS Identifying No: AVS-002883
Contractor's Identifying Code No: VN-I-106
Final Report Reference: Scheme 9

(vii) 2-(2-Hydroxyethyl)-9-(β-D-ribofuranosyl)hypoxanthine or
2-(2-Hydroxyethyl)inosine



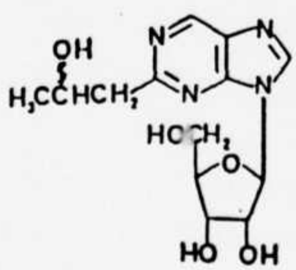
AVS Identifying No: AVS-002884
Contractor's Identifying Code No: VN-I-107
Final Report Reference: Scheme 11

(viii) 2-Acetonyl-9-(β-D-ribofuranosyl)purine or 2-Acetonylnebularine



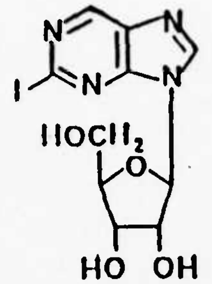
AVS Identifying No: AVS-003039
Contractor's Identifying Code No: VN-I-108
Final Report Reference: Scheme 6

(ix) 2-(2-Hydroxypropyl)-9-(β-D-ribofuranosyl)purine or
2-(2-Hydroxypropyl)nebularine



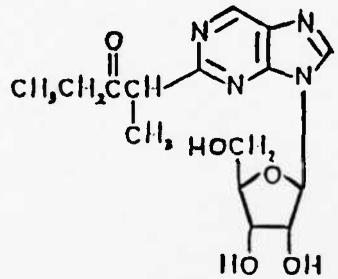
AVS Identifying No: AVS-003582
Contractor's Identifying Code No: VN-I-109
Final Report Reference: Scheme 6

(x) 2-Iodo-9-(β-D-ribofuranosyl)purine or 2-Iodonebularine



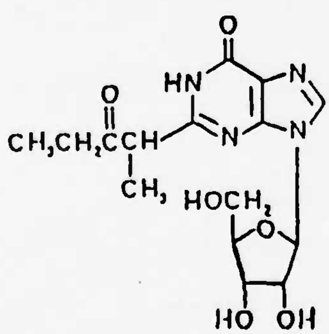
AVS Identifying No: AVS-003923
Contractor's Identifying Code No: VN-I-110
Final Report Reference: Page 11

(xii) 2-(1-Methyl-2-oxobutyl)-9-(β-D-ribofuranosyl)purine



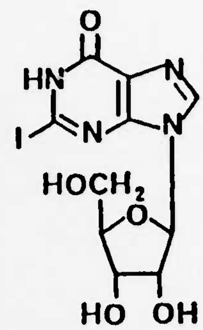
AVS Identifying No: AVS-003924
Contractor's Identifying Code No: VN-I-111
Final Report Reference: Scheme 13

(xii) 2-(1-Methyl-2-oxobutyl)-9-(β-D-ribofuranosyl)hypoxanthine



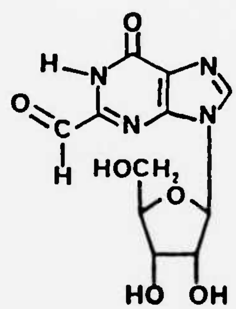
AVS Identifying No: AVS-003921
Contractor's Identifying Code No: VN-I-112
Final Report Reference: Scheme 12

(xiii) 2-Iodo-9-(β-D-ribofuranosyl)hypoxanthine or 2-Iodoinosine



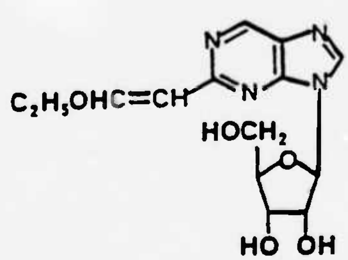
AVS Identifying No: AVS-003922
Contractor's Identifying Code No: VN-I-113
Final Report Reference: Page 11

(xiv) 2-Formylinosine



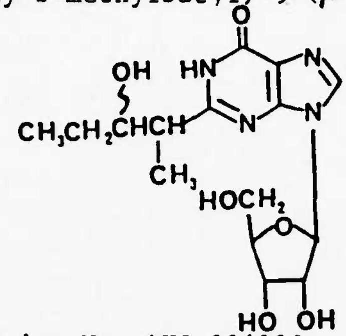
AVS Identifying No: AVS-004094
Contractor's Identifying Code No: VN-I-114
Final Report Reference: Scheme 16

(xv) 2-(E-2-Ethoxyvinyl)nebularine



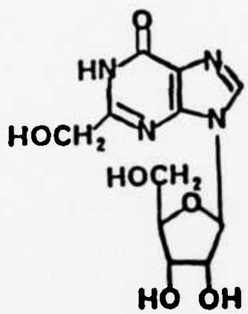
AVS Identifying No: AVS-004095
Contractor's Identifying No: VN-I-115
Final Report Reference: Scheme 15

(xvi) 2-(2-Hydroxy-1-methylbutyl)-9-(β-D-ribofuranosyl)hypoxanthine



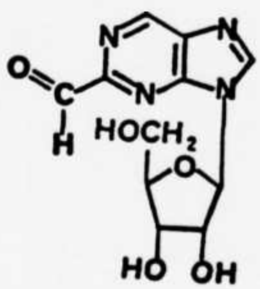
AVS Identifying No: AVS-004109
Contractor's Identifying Code No: VN-I-116
Final Report Reference: Scheme 19

(xvii) 2-Hydroxymethylinosine



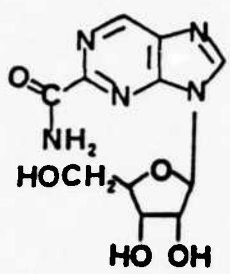
AVS Identifying No: AVS-004232
Contractor's Identifying Code No: VN-I-117
Final Report Reference: Scheme 17

(xviii) 2-Formylnebularine



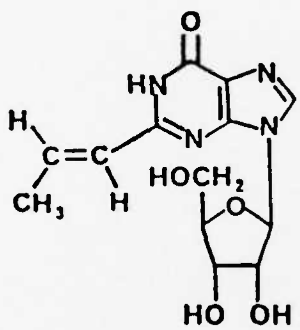
AVS Identifying No: AVS-004233
Contractor's Identifying Code No: VN-I-118
Final Report Reference: Scheme 18

(xix) 2-Carboxamidonebularine



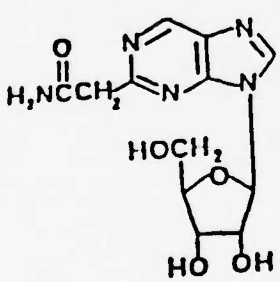
AVS Identifying No: AVS-004528
Contractor's Identifying Code No: VN-I-119
Final Report Reference: Scheme 20

(xx) E-2-(1-Propenyl)-9-(β-D-ribofuranosyl)hypoxanthine



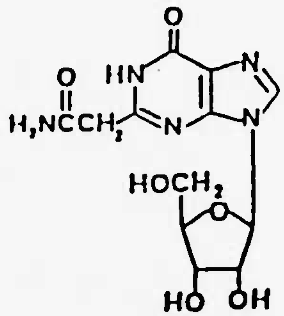
AVS Identifying No: AVS-004727
Contractor's Identifying Code No: VN-I-120
Final Report Reference: Scheme 23

(xxi) 2-Acetamidonebularine



AVS Identifying No: AVS-004928
Contractor's Identifying Code No: VN-I-121
Final Report Reference: Scheme 21

(xxii) 2-Acetamidoinosine



AVS Identifying No:
Contractor's Identifying Code No: VN-I-122
Final Report Reference:

7. Antiviral Screening Data:

AVS Identifying Number Contractor's Code Number	Antiviral Drug Screening Data
AVS-002159 VN-I-101	Very active, specific TI >1000 (*in vitro*, SF) HIV results not available Toxic in CCHF suckling mouse
AVS-002352 VN-I-102	Some activity against YF Not active against VSV, AD2, VV, HIV, SF, JE *in vitro* *in vivo* data not available
AVS-002716 VN-I-103	Some broad spectrum activity (*in vitro*) against YF,JE, AD2, VV, PT, RVF, not active against VEE, VSV, HIV *In vivo* data not available
AVS-002694 VN-I-104	Not active (*in vitro*) *In vivo* data not available
AVS-002695 VN-I-105	Not active (*in vitro*) *In vivo* data not available
AVS-002883 VN-I-106	Some activity against RVF Not active (*in vitro*) against other viruses HIV results not available *In vivo* data not available

AVS-002884 VN-I-107	Some activity against RVF Not active (in vitro) against other viruses HIV results not available In vivo data not available
AVS-003039 VN-I-108	Not active against AD2, JE, VSV, VV, RVF (in vitro) In vivo data not available
AVS-003582 VN-I-109	Not active against JBE, VV, AD2, VSV, RVF (in vitro) In vivo data not available
AVS-003923 VN-I-110	Not active against VSV, AD2, VV, SFS, VEE, JBE, YF, RVF (in vitro) In vivo data not available
AVS-003924 VN-I-111	Not active against VSV, VV, AD2, RVF (in vitro) In vivo data not available
AVS-003921 VN-I-112	Not active against VSV, AD2, VV, SFS, VEE, JBE, YF, RVF (in vitro) In vivo data not available
AVS-003922 VN-I-113	Not active against VSV, VV, AD2, JBE, VEE, SFS, YF, RVF (in vitro) In vivo data not available
AVS-004094 VN-I-114	Some activity against AD2, VSV Not active against JBE, PT, SFS, VEE, VV, YF (in vitro) In vivo data not available
AVS-004095 VN-I-115	Not active against VSV, AD2, VV, YF, VEE, PT, SFS, JBE (in vitro) In vivo data not available
AVS-004109 VN-I-116	Not active against VSV, AD2, VV, YF, VEE, PT, SFS, JBE (in vitro) In vivo data not available
AVS-004232 VN-I-117	Some activity against VV (in vitro) Not active against AD2, JBE, VSV In vivo data not available
AVS-004233 VN-I-118	Not active against AD2, JBE, VSV, VV (in vitro) In vivo data not available
AVS-004528 VN-I-119	Not active against AD2, VV, VSV (in vitro) In vivo data not available

AVS-004727 VN-I-120	Not active against AD2, VV (*in vitro*) Other data not available
AVS-004928 VN-I-121	Screening data not available
AVS- VN-I-122	Screening data not available

8. Bibliography of Publications, Patents, and Presentations:

(i) V. Nair, D. A. Young, and R. DeSilvia, Jr., 2-Halogenated Purine Nucleosides: Synthesis and Reactivity, Journal of Organic Chemistry, **1987**, 52, 1344 (4 copies furnished to SGRD-RMS).

(ii) V. Nair, D. A. Young, and R. DeSilvia, Jr., 2-Amino-9-(β-D-ribofuranosyl)purine. Photoinduced Reductive Dehalogenation: A General Approach to 2-Aminopurine and Related Systems, An Invited Article in "Nucleic Acid Chemistry", Part 4, Edited by L. B. Townsend and R. S. Tipson, 1987 (4 copies furnished to SGRD-RMS).

(iii) V. Nair, S. D. Chamberlain, R. DeSilvia, Jr., and G. S. Buenger, Synthetic Approaches to Rare 2-Substituted Purine Nucleosides, Nucleosides and Nucleotides, **1987**, 6, 229 (4 copies furnished to SGRD-RMS).

(iv) V. Nair and D. A. Young, Conformational Correlation of Purine Nucleosides by High-Field Carbon-13 NMR Data, Magnetic Resonance in Chemistry, **1987**, 25, 937 (4 copies furnished to SGRD-RMS).

(v) V. Nair, G. A. Turner, and S. D. Chamberlain, Novel Approaches to Functionalized Nucleosides via Palladium-Catalyzed Cross-Coupling with Organostannanes, Journal of the American Chemical Society, **1987**, 109, 7223 (4 copies furnished to SGRD-RMS).

(vi) V. Nair, Alkylated Inosines as Antiviral Agents, Patent Serial Number 67,498 filed with U. S. Patent Office, June 1987.

(vii) V. Nair, D. A. Young, S. D. Chamberlain, and G. S. Buenger, 2-Aminopurine Nucleosides: Synthesis, Biological Activity, and Reactivity, 21st Midwest Regional Meeting of the American Chemical Society, Kansas City, Missouri, November, 1986.

(viii) V. Nair, Synthetic Approaches to Rare 2-Substituted Purine Nucleosides, A Lecture at the 7th International Symposium on Nucleosides, Nucleotides, and their Biological Applications, Konstanz, Germany, 1986.

(ix) V. Nair, G. A. Turner, G. S. Buenger, and A. G. Lyons, Synthetic Approaches to New Biologically Active Purine Nucleosides, 194th National American Chemical Society Meeting, New Orleans, September 1987.

(x) V. Nair and G. S. Buenger, Novel 2-Substituted Purine Nucleosides, 99th Session of the Iowa Academy of Science, Grinnell, Iowa, April, 1987.

(xi) V. Nair and A. G. Lyons, Functionalization of Inosine, 99th Session of the Iowa Academy of Science, Grinnell, Iowa, April, 1987.

(xii) V. Nair, Rare 2-Substituted Purine Nucleosides, USAMRIID Department of Antiviral Studies Program Review, August, 1987.

(xiii) V. Nair, Eight Invited Research Seminars on "The Search for New Antiviral Compounds: Rare 2-Substituted Purine Nucleosides", presented at major Universities in Sydney, Melbourne, and Adelaide, Australia, July, 1987, as part of an Award as Distinguished Visiting Professor/Scholar.

(xiv) V. Nair, Planery Lecture, 8th International Symposium on Nucleosides, Nucleotides, and their Biological Applications, Perdido Beach, Alabama, October, 1988.

(xv) V. Nair and A. G. Lyons, Synthesis of Novel Purine Nucleoside Carboxaldehydes, 23rd Midwest Regional Meeting of the American Chemical Society, Iowa City, November, 1988.

(xvi) V. Nair, G. A. Turner, G. S. Buenger and S. D. Chamberlain, New Methodologies for the Synthesis of C-2 Functionalized Hypoxanthine Nucleosides, Journal of Organic Chemistry, **1988**, 53, 3051 (4 copies furnished to SGRD-RMS)

(xvii) V. Nair and G. S. Buenger, Rare Purine Nucleosides: Congeners of the Antibiotic, Nebularine, Synthesis, **1988**, 848 (4 copies furnished to SGRD-RMS)

(xviii) V. Nair and A. G. Lyons, Novel Unsaturated Purine Nucleosides, Journal of Organic Chemistry, **1989**, In Press (4 copies furnished to SGRD-RMS).

(xix) V. Nair and B. J. Hettrick, Sulfone of the Antibiotic, Nebularine: Synthesis and Conversion to Novel Analogues of Nebularine, Tetrahedron, **1988**, 44, 7001 (4 copies furnished to SGRD-RMS)

(xx) V. Nair, Development of Methodologies for the Strategic Modification of Purine Ribonucleoside Systems, Nucleoside and Nucleotides, **1989**, In Press (Proceedings of the 8th International Round Table Symposium on Nucleosides, Nucleotides, and their Biological Applications)

9. Personnel Supported:

Stanley D. Chamberlain, Ph.D. Degree, December 1986.
Raymond DeSilvia, Jr., M.S. Degree, August 1986 (Deceased).
Gregory A. Turner, Ph.D. Degree, May, 1987
Greg S. Buenger, Ph.D. Degree (Expected May 1989)
Arthur G. Lyons, Ph.D. Degree (Expected May 1989)
Brian J. Hettrick, M. S. Degree, May, 1988

10. Summary:

In the over three years on this contract, we have had considerable success in our synthetic work and a total of twenty-two rare 2-substituted purine nucleosides were synthesized, purified, characterized, and submitted to the Department of Antiviral Studies with supporting data. Although screening data are not complete as yet on several of the compounds submitted, some very interesting and positive data have been received. One compound (2-acetonylinosine, AVS-00159) has been found to have very high activity (TI > 1000) against the Sandfly Fever Virus (Phlebovirus). Another compound (2-vinylinosine, AVS-002716) has been found to have low but broad spectrum activity against a number of RNA viruses. Two other compounds (AVS-002883 and AVS-002884) have shown some activity against the Rift Valley Fever Virus, and still another (AVS-002352) has shown activity against the Yellow Fever Virus. 2-Formyinosine (AVS-004094) has shown some activity against Type 2 Adenovirus and 2-hydroxymethylinosine (AVS-004232) has shown activity against the Vaccinia Virus. Ten publications and a patent have arisen directly from this work. Various aspects of the synthetic work have also been presented as invited and contributed papers (including a planery lecture) and research seminars at regional, national, and international scientific meetings and occasions.